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Thanks,

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Rapid Induction of Vascular Endothelial Growth Factor Gene Expression After Transient Middle Cerebral Artery Occlusion in Rats

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Background and Purpose Vascular endothelial growth factor (VEGF) is a mitogen for endothelial cells and also has the potential to increase vascular permeability. Therefore, it may contribute to the recovery of brain cells from ischemic insult through potentiating neovascularization or may exacerbate brain damage by forming brain edema. However, the exact role of this protein in cerebral ischemia is not fully understood. We investigated temporal, spatial, and cellular profiles of the induction of VEGF gene expression after transient focal cerebral ischemia at both mRNA and protein levels.

Methods We used a transient middle cerebral artery (MCA) occlusion model. Northern blot analysis was performed to assess the chronological pattern of induction and the impact of length of ischemia on mRNA expression. Western blot analysis was performed to ensure the selective detection of immunoreactive VEGF with an antibody. Temporal, spatial, and cellular changes of immunohistochemical VEGF expression were compared with different periods of reperfusion from 1 hour to 7 days after transient MCA occlusion.

Results (1) Northern blot analysis revealed no detectable VEGF mRNA in the control brains. The mRNA became evident at 1 hour after reperfusion, peaked at 3 hours, and then decreased. The length of ischemia from 1 to 3 hours made no differences in the degree and temporal profile of the subsequent induction of VEGF mRNA. (2) Western blot analysis showed no band in the control brain, but two bands with molecular weights of 38 and 45 kD, corresponding to $VEGF_{121}$ and $VEGF_{165}$, were induced at 1 hour of reperfusion, peaked at 3 hours of reperfusion, and then decayed. (3) Neurons in the cerebral cortex of the MCA territory expressed VEGF at 1 hour after reperfusion with a peak at 3 hours and then diminished by 1 day. Pial cells of the MCA territory also expressed immunoreactive VEGF from 1 hour of reperfusion that was sustained until 3 to 7 days after reperfusion.

Conclusions Rapid induction of VEGF gene expression after transient MCA occlusion was demonstrated at both mRNA and protein levels. Cortical neurons and pial cells were the source of VEGF production in this model, but the temporal profiles of the induction between these cells were different. The early but dissociative induction of VEGF between neuronal and pial cells suggests different roles of the protein in their cells after transient MCA occlusion. (*Stroke*. **1997;28:2039-2044.**)

Key Words • angiogenesis • cerebral ischemia • rats

Vascular endothelial growth factor, also known as vascular permeability factor, is a dimeric glycoprotein that is mitogenic for endothelial cells and has the potential to increase vascular permeability. By alternative splicing, four different isoforms exist in vivo: $VEGF_{206}$, $VEGF_{189}$, $VEGF_{165}$, and $VEGF_{121}$.[1-3] The polypeptides of $VEGF_{206}$ and $VEGF_{189}$ bind well to heparin-containing proteoglycans of the extracellular matrix. However, $VEGF_{165}$ and $VEGF_{121}$ do not bind to proteoglycans and thus act as diffusible factors. Although VEGF is normally expressed in ependyma,[4] choroid plexus, and granule cells in cerebellum,[5] the role in normal brain is not well characterized.

It is well known that ischemic stroke causes active angiogenesis, particularly in the ischemic penumbra, which correlates with longer survival in humans.[6] Therefore, it is speculated that VEGF plays an important role in the recovery of cerebral infarct. On the other hand, VEGF increases vascular permeability and is involved in the development of brain edema in patients with brain tumor.[7] Thus, VEGF might also be harmful in cases of ischemic stroke by potentiating brain edema. Furthermore, VEGF has additional important roles in relaxing vascular smooth muscle cells,[8,9] preventing apoptosis of endothelial cells,[10] and inducing chemotaxis of monocytes under pathological conditions.[4] When one considers these possible ambivalent effects of VEGF under pathological brain conditions such as cerebral ischemia, it is important to clarify how the VEGF gene is expressed in cerebral ischemia.

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Previous studies revealed that hypoxia induces production of VEGF in cultured glial[11] and endothelial[12,13] cells. A recent study of permanent MCA occlusion in rats showed a late increase of VEGF protein in neurons, astrocytes, and macrophages with a peak at 1 day in the ischemic core and at 7 days in the penumbra.[14] However, the effect of reperfusion on gene expression has not been reported. Unlike the case of permanent MCA occlusion, mild transient ischemia causes brain edema without development of cerebral necrosis.[15] Thus, there may be a difference in the temporal profiles and the roles of VEGF between reperfused and permanent ischemic

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Selected Abbreviations and Acronyms

CBF	= cerebral blood flow
MCA	= middle cerebral artery
PBS	= phosphate-buffered saline
SDS	= sodium dodecyl sulfate
SSC	= standard saline citrate
VEGF	= vascular endothelial growth factor

brains. We examined the induction of the VEGF gene at both mRNA and protein levels after mild transient MCA occlusion in rats. A rapid and dissociative induction of VEGF between neuronal and pial cells of the MCA territory was found after transient ischemia.

Materials and Methods

Animal Model

This experiment was approved by the Animal Committee of Tohoku University School of Medicine.

Sixty-seven male Wistar rats weighing 250 to 280 g were used. The rats were lightly anesthetized by inhalation of a 69%/30% (vol/vol) mixture of nitrous oxide/oxygen and 1% halothane during surgical preparation. A midline neck incision was made, and the right common carotid artery was exposed; inhalation of anesthetics was then stopped. When the animal began to regain consciousness, the right MCA was occluded by insertion of nylon thread through the common carotid artery, as described in our previous report.[16] During these procedures, body temperature was monitored with a rectal probe and maintained at 37±0.3°C with the use of a heating pad. The restoration of CBF was accomplished by removal of the nylon thread under light anesthesia with diethyl ether. Between MCA occlusion and CBF restoration and after CBF restoration, the surgical incision was closed, and the animals were allowed free access to water and food at ambient temperature (21°C to 23°C).

The animals were divided into three experimental groups: group A, for investigation of mRNA induction (n=47); group B, for Western blot study (n=8); and group C, for immunohistochemical analysis (n=12).

Group A

To investigate how the length of ischemia influences the degree and duration of mRNA induction for VEGF, 1, 2, or 3 hours of ischemia was imposed on each animal. The animals were decapitated under deep anesthesia with diethyl ether 1 or 3 hours or 1, 3, or 7 days after the restoration of CBF (n=3 in each time point for each ischemic duration). Brain samples from two sham control rats were obtained; these rats underwent a surgical procedure until the carotid artery was exposed, but the MCA was not occluded. Cerebral cortical samples of the right MCA territory were quickly obtained and were immediately frozen in powdered dry ice. They were temporarily kept at −70°C until the extraction of RNA.

Group B

The duration of ischemia was 90 minutes for these animals. After 1 or 3 hours or 1 day of reperfusion, cerebral cortical samples of the MCA territory were obtained (n=2) and kept as noted above for group A. Sham controls samples (n=2) were also obtained.

Group C

For immunohistochemical analysis, the animals were deeply anesthetized with pentobarbital (50 mg/kg IP). Both deep cervical veins were cut at 1 or 3 hours or 1, 3, or 7 days of reperfusion after 90 minutes of transient ischemia (n=2). The brain was perfused with heparinized physiological saline through the left cardiac ventricle at 110 mm Hg pressure until colorless perfusion fluid was obtained from the cervical veins, followed by an additional 200 mL of cold 4% paraformaldehyde in PBS. The brain was removed and stored in the same fixative at 4°C for 48 hours and embedded in paraffin. Sham control animals (n=2) underwent the same procedure except for MCA occlusion and reperfusion.

Northern Blot Analysis

Total RNAs were extracted from the cerebral cortical samples of Group A animals by the guanidinium thiocyanate method. Northern transfer and hybridization were essentially the same as described in our previous report.[15] In brief, 20 μg of total RNA was electrophoresed in a 1.2% agarose gel containing 10% formaldehyde and transferred overnight onto a nylon membrane (Hybond N, Amersham). RNA was fixed with 1200 μJ ultraviolet (Stratalinker, Stratagene), and then prehybridized. Signals for VEGF mRNA were detected with an enhanced chemiluminescence method (direct nucleic acid labeling and detection systems, Amersham) according to our previous report.[17] A cDNA clone of rat VEGF, a gift from Dr E. Hashimoto (Fourth Department of Internal Medicine, School of Medicine, University of Tokyo), was used as a probe. The specificity of this cDNA had been established elsewhere.[18] Then 0.6 kb of the insert was released, and the insert DNA was labeled with horseradish peroxidase and hybridized at 42°C for 20 hours in a hybridization solution of the kit. After hybridization, the membrane was washed with 2×SSC (1× is 150 mmol/L NaCl, 15 mmol/L trisodium citrate, pH 7.0), 0.4% SDS twice, 1×SSC with 0.2% SDS once, and 20×SSC without SDS twice. Detection reagents in the kit were added to the blots on the face carrying the RNA. The membrane was exposed to an x-ray film for 30 minutes at room temperature and later developed. Hybridization experiment was repeated with the use of a tubulin DNA probe.[14]

Western Blot Analysis

The tissue samples were homogenized in a lysis buffer (0.1 mol/L NaCl, 0.01 mol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, and 1 μg/mL aprotinin). Then the homogenates were centrifuged at 7000*g* for 15 minutes at 4°C, and the supernatents were used as protein samples. Assays to determine the protein concentration of the supernatants were subsequently performed by comparison with a known concentration of bovine serum albumin with a kit (Micro BCA, Pierce). SDS-gel electrophoresis was performed in a 10% polyacrylamide gel under nonreducing conditions. Lysates equivalent to 40 μg of protein from each samples were run on the gel for 90 minutes at 20 mA, together with a size marker (rainbow colored protein, Amersham). The electrophoresis running buffer contained 25 mmol/L Tris base, 250 mmol/L glycine, and 0.1% SDS. The proteins on the gel were subsequently transferred to the polyvinylidene fluoride transfer membrane (Micron Separations Inc) in a buffer containing 20% methanol, 39 mmol/L glycine, 48 mmol/L Tris base, and 0.4% SDS.

After the transfer, the membrane was placed in 1% powdered milk in Tween PBS (PBS with 0.1% Tween 20) at 4°C for 16 hours to block nonspecific binding. Then it was washed with Tween PBS three times and incubated with mouse monoclonal antibody for VEGF (2E1, IBL) at 1:20 dilution in Tween PBS at 4°C for 20 hours. After it was washed with Tween PBS three times, the membrane was incubated with biotinylated anti-mouse IgG (Vectastain, PK-6102, Vector Laboratories) at 1:200 dilution in Tween PBS for 90 minutes at room temperature. It was washed with PBS for three times and incubated with biotin-avidin-peroxidase complex solution with the use of a kit (PK-6102, Vector Laboratories) for 60 minutes. The membrane was then developed with 3,3′-diaminobenzidine tetrahydrochloride (0.5 mg/mL in 50 mmol/L Tris-HCl, pH 7.5) in the presence of 0.02% H_2O_2. The membrane was then washed in distilled water and air dried. To ascertain specific

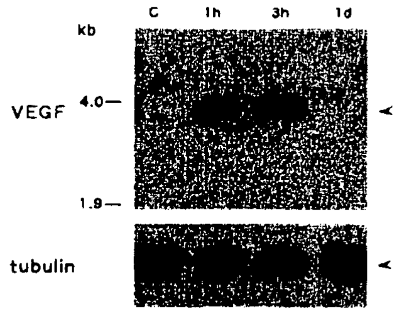

FIG 1. Northern blots demonstrate time course of VEGF and tubulin mRNA levels. In the control lane, no band for VEGF mRNA is detectable (top), with subsequent induction from 1 hour of reperfusion after 2 hours of transient MCA occlusion. The induction became maximal at 3 hours and returned to the control level by 1 day of reperfusion (arrowhead). The levels of tubulin mRNA (arrowhead) do not show significant change (bottom).

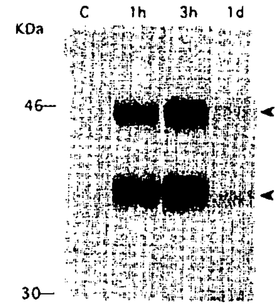

FIG 2. Western blots demonstrate induction of two isoforms of VEGF protein. Immunoreactive VEGF was scarce in the control brain. With reperfusion after 90 minutes of transient ischemia, two bands of molecular weight at 38 and 45 kD (arrowheads) became evident at 1 hour with a peak at 3 hours and decreased by 1 day. The smaller and larger bands correspond to $VEGF_{121}$ and $VEGF_{165}$, respectively.

binding of the antibody for the protein, another filter was stained in a similar way without the first antibody.

Immunohistochemical Analysis

Coronal sections at the caudate level were cut with 1 μm thickness from the paraffinized samples of group C animals and mounted on standard glass slides. Deparaffinization was achieved by treating the specimens in xylene and subsequent ethanol, followed by a rinse in PBS. After they were blocked with 10% normal horse serum for 2 hours, the slides were washed in PBS and incubated with a mouse monoclonal antibody against VEGF at 1:50 dilution in 10% normal horse serum and 0.3% Triton X-100 for 16 hours at 4°C. Endogenous peroxidase activity was quenched by exposing the slides to 0.3% H_2O_2 and 10% methanol for 20 minutes. The slides were then washed and incubated for 3 hours with biotinylated anti-mouse IgG at 1:200 dilution in PBS containing 0.018% normal horse serum. Subsequently they were incubated with avidin-biotin-horseradish peroxidase complex for 30 minutes and then developed with the use of diaminobenzidine as a color substrate. The reaction was stopped by washing the slides in distilled water. To ascertain specific binding of the antibody for the protein, a set of brain sections was stained in a similar way without the first antibody. The staining was categorized into four grades, as follows: no staining (−), slight staining (±), moderate staining (+), or dense staining (2+).

Results

Northern Blot Analysis

As shown in Fig 1 (top panel), there was no band in the control brain for VEGF mRNA. After 2 hours of transient MCA occlusion, an increase of VEGF mRNA level became evident at 1 hour after the CBF was restored, peaked at 3 hours, and decreased to baseline level by 1 day of reperfusion. No bands were detectable at 3 and 7 days after reperfusion (not shown). Variation of length of ischemia from 1 to 3 hours made no difference in the degree of mRNA induction (not shown). The temporal profile of mRNA induction was also the same regardless of the length of ischemia (not shown). The size of VEGF mRNA detected in this study was 3.9 kb, which is compatible with a previous report.[18] On the other hand, the levels of tubulin mRNA did not change (Fig 1, bottom panel). Triplicate samples in each time point for each duration of ischemia showed reproducible results.

Western Blot Analysis

Since the length of ischemia showed no essential difference in the degree and temporal changes of VEGF gene expression, the following studies were performed with constant duration of ischemia for 90 minutes. As shown in Fig 2, immunoreactive VEGF was barely detectable in the control brain, but brain lysate at 1 hour of reperfusion revealed two bands. These bands became more distinct in the lysate at 3 hours of reperfusion and decreased at 1 day. The two bands correspond to 38 and 45 kD, the former more strongly detected. These bands correspond to $VEGF_{121}$ and $VEGF_{165}$, respectively. A blot without the first antibody did not show any bands. Duplicated samples in each time point showed reproducible results.

Immunohistochemical Analysis

Brain sections without the first antibody showed no staining. With the antibody, immunoreactivity of VEGF was normally present in the ependymal cells (data not shown), while there was no detectable immunoreactive VEGF in neurons, glial cells, vascular endothelial cells, or pial cells in control brains (Fig 3a).

Our preliminary studies revealed that cerebral tissue necrosis occurred after 2 or 3 hours of ischemia, but no discernible tissue necrosis occurred under 90 minutes or 1 hour of transient ischemia. These results were essentially the same as those in our recent report.[19]

At 1 hour of reperfusion after transient MCA occlusion, immunoreactive VEGF became detected in the cytoplasm of a part of neurons in the cerebral cortex of the MCA territory (Fig 3b, arrowheads). Pyramidal neurons in the third to fifth layers of the cerebral cortices were selectively stained. There was no expression in neurons of caudate nucleus even within the MCA territory. At 3 hours after reperfusion, the expression of VEGF in neurons of the MCA area became more intense (Fig 3c). The staining then greatly decreased at 1 day of reperfusion, with very slight staining in only a few neurons (Fig 3d, arrowhead). No more immunoreactive VEGF was found in neurons at 3 and 7 days after reperfusion. No immunoreactive VEGF in vascular endothelial or glial cells was documented at any stage of reperfusion.

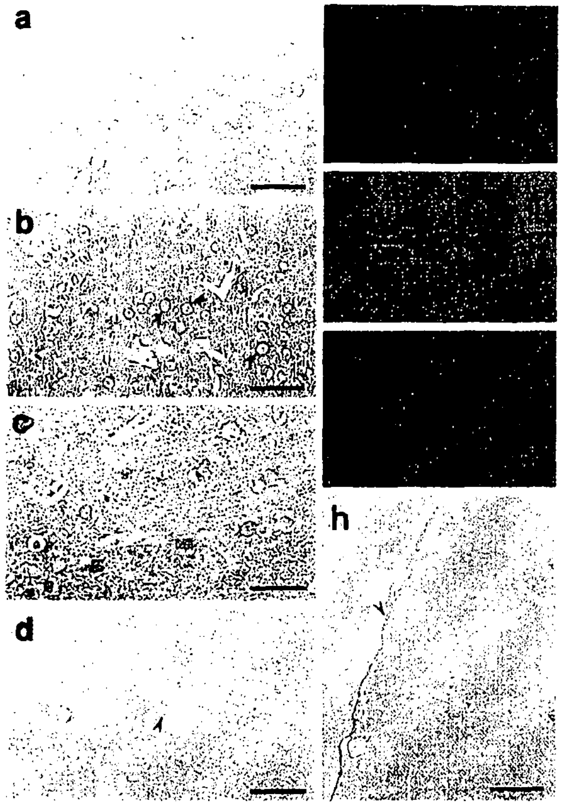

FIG 3. Representative photographs of immunohistochemistry for VEGF. Panels a through d show changes of immunoreactive VEGF in cerebral cortical neurons, and panels e through h show changes in pia mater. In contrast to control (a), note weak staining in a few neurons at 1 hour of reperfusion (b, arrowheads), with increasing staining at 3 hours in many neurons (c). Most neurons show negative staining, except for a very few neurons at 1 day (d, arrowhead). In addition, note no staining in the pia mater of control brain (e, arrowhead) with evident staining at 3 hours (f, arrowhead) lasting 3 days (g, arrowhead). Pia mater in left lower side of black arrowhead in panel h shows MCA territory, and that in right upper side shows the anterior carotid artery territory. Note selective staining of pia mater in the MCA territory and staining of neurons in the marginal zone (h, white arrowheads). Bar=100 μm in panels a, b, d, e, f, and g; bar=50 μm in panel c; bar=400 μm in panel h.

Pial cells of the MCA area showed no immunoreactive VEGF in control brains (Fig 3e, arrowhead). However, pial cells became stained from 1 hour after reperfusion, with a peak at 3 hours (Fig 3f). Unlike the temporal changes in cortical neurons, immunoreactivity in pial cells was sustained until 3 to 7 days after reperfusion (Fig 3g, arrowhead). The expression of immunoreactive VEGF was restricted in the MCA territory (Fig 3h, left lower side from black arrowhead), in contrast to the pia mater in the ipsilateral anterior cerebral artery area showing a negative immunoreactivity for VEGF (Fig 3h, right upper side from black arrowhead). Sections at 1 hour of reperfusion showed staining in neurons of the cerebral cortex in MCA territory and its marginal zone (Fig 3h, white arrowheads). The temporal profiles and the staining properties of VEGF expression for each cell type of each animal are reproducible and are summarized in the Table, illustrating the difference in chronological sequence between neurons and pia mater.

Temporal Profiles of Immunoreactive VEGF Expression in Neuron and Pia Mater of the MCA Region After Transient Ischemia

		Time After Reperfusion				
	Control	1 h	3 h	1 d	3 d	7 d
Neuron	–	+	2+	±	–	–
	–	+	2+	–	–	–
Pia mater	–	+	2+	+	±	±
	–	+	2+	+	±	–

The symbols –, ±, +, and 2+ represent no, slight, moderate, and dense immunoreactivity for VEGF, respectively, in each animal.

Discussion

Northern blot analysis revealed a rapid induction of VEGF mRNA as early as 1 hour after reperfusion. In addition, the decrease of the VEGF mRNA level was also rapid, and VEGF mRNA was no longer detectable at 1 day after reperfusion (Fig 1). However, previous reports showed late onset and sustained expression of VEGF in the following. In vivo, the induction was slower in pigmented epithelium of mouse retina under hypoxic conditions.[20] Furthermore, in a permanent MCA occlusion model in rats, the induction of VEGF protein became evident only after 18 hours of ischemia and lasted 14 days.[14] In vitro study of cardiac myocyte[21] and human Müller cells[22] also showed a slower induction of VEGF in hypoxia. Although the reason for this chronological difference between the present result and the previous reports is yet to be resolved, Banai et al[23] proposed the possibility that ischemia (ie, reduction of oxygen and glucose levels) might not be a prerequisite for VEGF induction. Reoxygenation of damaged tissue with reperfusion may be more important for the rapid expression of the VEGF gene. In fact, in an experiment with rat perfused heart, VEGF mRNA became evident as early as 15 minutes after reperfusion following 10 minutes of ischemia.[18] The same experiment also showed that longer duration of ischemia did not increase the degree of VEGF mRNA induction.[18] This seems to be compatible with our findings that duration of ischemia varying from 1 to 3 hours made no difference in the degree of induction and exhibited the ame temporal profiles.

By immunohistochemical analysis, neurons in the cerebral cortex and pial cells expressed immunoreactive VEGF after ischemic insult (Fig 3). Although previous studies demonstrated that VEGF gene was induced in glial cells,[24] glia-derived tumor cells,[25,26] cerebral endothelial cells,[12,13] and pericytes[27] under hypoxic conditions in vitro and in glial cells in permanent MCA occlusion in vivo,[14] glial cells and vascular endothelial cells did not express the VEGF gene in this experiment. Sensitivity for inducing the VEGF gene in neuronal, glial, and pial cells may be different under conditions of hypoxia/ischemia or ischemia/reperfusion. It is intriguing that neurons in the third to fifth layers of cerebral cortex selectively expressed immunoreactive VEGF (Fig 3a through 3d). These neurons are known to be selectively vulnerable to ischemic insults.[28] However, caudate neurons also vulnerable to ischemia[28] did not express the gene in the present study. The reason for this dissociation is yet to be clarified. This is the first report of VEGF gene

expression in pial cells. VEGF is a mitogen for endothelial cells[29] and also has the potential to increase vascular permeability.[30,31] Neovascularization usually occurs in brain infarct,[6] and the proliferation of endothelial cells begins at 2 to 5 days after the ischemic insult and continues for months.[6,32] Therefore, the sustained expression of the VEGF gene in the pia mater after ischemia (Fig 3e through 3h) suggests its role in new vessel formation in the affected area. On the other hand, the rapid and transient induction of VEGF in neurons (Fig 3a through 3d) seems to be related to other functions, since transient induction of growth factor is usually insufficient for new vessel formation.[33,34] One possibility is the protection of the vascular system against ischemic insult, because VEGF protects capillary endothelial cells from apoptotic cell death.[10] VEGF also relaxes vascular smooth muscle cell[8,9] and therefore might contribute to the protection of brain tissue from ischemia.

In this experiment constitutive expression of VEGF in ependymal cells did not affect the control level of mRNA (Fig 1) because tissue samples were obtained from the cerebral cortex of the MCA territory and did not contain the above cells. On the other hand, it seems contradictory that the pia mater expressed VEGF protein until 3 to 7 days of reperfusion (Fig 3g) without an evident signal of mRNA in Northern blot (Fig 1). This may be due to differences in sensitivity for detecting mRNA and protein. The bulk of pia mater might be too small to detect VEGF mRNA in Northern blot.

The effect of VEGF on increases in vascular permeability might worsen brain edema caused by cerebral ischemia. However, brain edema associated with mild cerebral ischemia is not mainly induced by an increase in vascular permeability (vasogenic edema)[35,36] but rather is due to cellular damage (cytotoxic edema).[37] Although expression of VEGF and its ability to increase vascular permeability were inhibited by dexamethasone,[38] dexamethasone was not effective in reducing ischemic brain edema.[37] Furthermore, the peak of brain edema in this model was 12 hours to 1 day,[15] while the induction of VEGF gene was much more earlier at 1 to 3 hours (Figs 1 and 2). Thus, the early induction and disappearance of VEGF gene in neurons may not be directly involved in the brain edema formation in the present model.

Western blot analysis showed induction of two isoforms, $VEGF_{165}$ and $VEGF_{121}$, in rat brain after ischemia (Fig 2). Since this antibody was raised against full-length VEGF protein, the possibility that it does not detect $VEGF_{206}$ and $VEGF_{189}$ even if these isoforms were present cannot be excluded. However, a previous study suggested that $VEGF_{206}$ and $VEGF_{189}$ were relatively rare in rat brain.[3] Furthermore, in a permanent MCA occlusion model in rats, only $VEGF_{165}$ and $VEGF_{121}$ were induced in the brain.[14] It is therefore likely that only two isoforms, $VEGF_{165}$ and $VEGF_{121}$, were induced in the brain in this experiment. It has been reported that $VEGF_{206}$ and $VEGF_{189}$, which bind to proteoglycans, lack mitogenic activity for vascular endothelial cells.[5] It is therefore probable that $VEGF_{165}$ and $VEGF_{121}$ play more important roles in ischemic brains as diffusible isoforms of VEGF.

In animal models of hindlimb ischemia[39,40] and myocardial infarction,[23] injection of VEGF was demonstrated to be therapeutically useful by potentiating angiogenesis and enhancing collateral blood flow. It has been reported that replication-deficient adenovirus as a vector of VEGF gene infected vascular endothelial cells and induced expression of VEGF.[41] An injection of VEGF or gene therapy with VEGF might also be effective for reducing brain damage due to ischemic insults. In the present study, rapid expression with dissociative decrease of VEGF induction in different cell types was shown in MCA areas after transient ischemia. The different temporal profiles might be related to different roles in cerebral ischemia. Therefore, it is important to clarify the influence of VEGF on cerebral circulation, brain edema, and cerebral neovascularization under ischemic and/or reperfused conditions.

Acknowledgments

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Editorial Comment

Vascular endothelial growth factor (VEGF), a member of the platelet-derived growth factor (PDGF) superfamily, is one of several identified angiogenic growth factors. VEGF is an endothelial mitogen, and induces new capillary formation (angiogenesis) in vivo. VEGF also increases vascular permeability.

Previous reports have shown upregulation of VEGF following focal brain infarction.[1] What's new in the current report is the description of two phases of VEGF expression following reversible ischemia: a brief initial phase (1 to 24 hours) occurring in neurons, and a longer persistent phase (1 hour to 7 days) occurring in pial cells.

The significance of these two phases of VEGF expression is unclear. It is possible, for example, that the first phase might contribute to increased vascular permeability and resultant edema formation, whereas the second phase might contribute to the angiogenesis that occurs during the first few days after stroke. Further work will be required to address these issues.

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